Reduced **infancy and** childhood epilepsy following hypothermia-treated neonatal encephalopathy

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Summary

Objective

To investigate what proportion of a regional cohort of cooled infants with neonatal encephalopathy, develop epilepsy **(ILAE-definition and AED)** up to 8 years of age.

Methods

From 2006-2013, 151 infants, with perinatal asphyxia underwent 72 hours cooling. Clinical and **aEEG with single-channel EEG** verified neonatal seizures were treated with anti-epileptic drugs (AEDs). Brain MRI was assessed using a 0-11 severity score.

Post-neonatal seizures, epilepsy-rates and AED treatments were documented. 134 survivors were assessed at 18-24 months; adverse outcome was defined as death or Bayley-III composite Cognition/Language or Motor scores <85 and/or severe cerebral palsy or severely reduced vision/hearing. Epilepsy-rates in 103 children age 4-8 years **were also documented.**

Results

aEEG confirmed seizures **occurred** pre-cooling in 77/151(57%) **neonates; 48%** seized during and/or after cooling and received AEDs. Only one infant was discharged on AEDs. At 18-24 months one-third of infants had adverse outcome including 11% mortality. At 2 years, 8(6%) infants had an epilepsy diagnosis **(ILEA definition),** of whom 3(2%) received AEDs. **Of the** 103 **4-8 year olds**, 14(13%) **had** developed epilepsy, **with** 7(7%) receiving AEDs. Infants**/children** on AEDs had higher MRI scores than those not on AEDs, (median (IQR) 9(8-11) vs. 2(0-4)) and poorer outcomes. **Nine of 14 children with epilepsy** had CP(64%) compared to 13/120(11%) without epilepsy **and** 10/14(71%) **children with epilepsy** had adverse outcomes versus 23/120(19%) survivors without epilepsy. The number of different AEDs given to control neonatal seizures, aEEG severity pre-cooling and MRI scores predicted childhood epilepsy.

Significance

We report, in a **regional cohort of infants cooled for perinatal asphyxia**, 6% with epilepsy at 2 years (2% on AEDs) increasing to 13%(7% on AEDs) at early school age. These AED rates are much lower **than reported in the cooling trials even adjusting for our cohort´s milder asphyxia**. Long-term follow-up is needed to document final epilepsy-rates.

Key words: Epilepsy, seizure, ILAE, AED, hypothermia, hypoxia-ischemia, newborn, childhood

Key Points

1. 151 infants with moderate or severe perinatal asphyxia **treated with** therapeutic hypothermia, 75% had **neonatal** seizures confirmed on single-channel electroencephalogram (EEG) **and** amplitude integrated EEG **before**, during **or after cooling**.
2. Seizures occurred in 43% of infants during hypothermia treatment; only 1 of 134 survivors was still on antiepileptic drugs at discharge.
3. The epilepsy rate **(ILAE definition)** increased **from 6% at 2 years to 13% at 4-8 years with 2% and 7% respectively on medication**.
4. Of children with epilepsy, 64% had cerebral palsy as compared to 11% **with CP** among the 120 **children** without epilepsy in the cohort.

Introduction

Childhood epilepsy affects cognitive performance, life quality and life expectancy. The prevalence of epilepsy requiring anti-epileptic drugs (AEDs) in patients of any age is about 0.97% in the UK[1] while the overall rate of seizures under the age of four is 0.2%[1]. In infants who suffer hypoxic-ischemic encephalopathy (HIE)[2] the post-neonatal epilepsy rate at 18 months when defined as needing regular AEDs, is reported to be 15-16%[3-6] both in infants who did and did not receive therapeutic hypothermia (TH). At school age, rates are reported to be 10%-16%[7]. **Recently the International League Against Epilepsy (ILAE) definition of epilepsy was published[8], namely two or more unprovoked seizures at least 24 hours apart or a formal epilepsy syndrome diagnosis. In our paper we present epilepsy rates both according to the definition used in earlier HIE follow-up papers and according to ILAE.**

The relatively novel treatment of TH, has significantly reduced the relative risk of the combined adverse outcome, death and disability in infants with HIE at birth; 0.75 (95% confidence interval, CI, 0.68-0.83)[9]. Cooling to core temperature 33.5˚C for 72h after moderate or severe perinatal asphyxia is now standard of care in developed countries. Our centre has cooled infants as a part of feasibility studies, randomised trials or registered follow-up programs since 1998[3; 5; 10-12]. We introduced TH as a standard treatment in December 2006 when recruitment to the TOBY trial[5] ended. The aim of our current study was to examine the incidence of early childhood seizures in a cohort of cooled infants treated in a manner representative of clinical cooling practices for perinatal asphyxia of mostly moderate severity and seizure management in a single regional tertiary referral centre in the UK.

Methods

This study has waiver consent and ethical permission **(09/H016/3)** to use prospectively collected anonymised clinical data from the Bristol cooling database

Inclusion and exclusion criteria for cooling

Our tertiary cooling centre serves 8 level II regional hospitals and 165 infants received TH between December 2006 and October 2013. Infants born at ≥36 gestational age (GA) were assessed as eligible for cooling using the three (A, B and C) CoolCap and later TOBY trial entry criteria[3; 5]; A: metabolic, B: **neurological** and C: modified amplitude integrated electroencephalographic **(aEEG)** criteria.
A: fulfilment of at least one of: 1) Apgar score ≤5 at 10min, 2) continued need for assisted ventilation 10min after birth, 3) pH <7.0 within the first hour after birth, 4) base deficit ≥16mmol/L within the first hour after birth.

B: clinical presentation of moderate or severe encephalopathy having reduced consciousness including being lethargic or stuporous, and in addition at least one of the following: hypotonia, abnormal reflexes, an absent or weak suck and clinical seizures.
C: Moderately or severely abnormal amplitude-integrated electroencephalogram (aEEG) background voltage pattern within 6h of birth: (i.e. moderate depression with lower band <5µV and upper band >5µV or severe depression with lower band <5µV and upper band <10µV[3; 13]) or **single-channel EEG** confirmed seizures lasting at least 3min within 1 hour with any background pattern. Since 2008, we also recruited encephalopathic infants using modified cooling entry criteria **for 20% of patients**[14] i.e. including infants with early postnatal collapse, major extracerebral or intracerebral or intraventricular haemorrhage, a diagnosis requiring major surgery, a cardiac diagnosis, mild prematurity (gestational age 34-36 weeks) or starting TH late from 6 to 12h after birth. **In the current paper,** 19% of infants who were cooled had these modified entry criteria. Exclusion criteria were infants <2nd weight centile for GA, on-going bleeding and very poor condition so treatment was deemed futile.

Cooling management

When cooling entry criteria A and B were fulfilled (usually within 1 hour of birth), passive cooling (removing any external heating and infant's **rectal temperatures closely monitored**) or active cooling (using cooling equipment) was initiated while aEEG monitoring was started. The **"worst"** aEEG **pattern seen lasting at least 30min** within 6h of birth **classified the infant for cooling** to 33.5°C for 72h **or not**. TH was maintained using either a servo-controlled body wrap circulated with water, (Criticool, Charter Kontron Ltd. Milton Keynes, UK n=145), a servo-controlled cooling mattress Tecotherm Neo (n=16, Inspiration Healthcare Ltd. Leicester, UK) or using a manually controlled head-cooling system at a rectal temperature of 34.5°C (CoolCap, Natus Medical Inc. Seattle, USA) n=4. All infants were sedated with morphine, typically starting at 20µg/kg/hour, the dose being adjusted according to clinical needs during TH and intensive care. This practice is based on experimental evidence demonstrating that cooling without sedation compromises the neuroprotective effect of cooling[15].

Seizures, aEEG monitoring and anti-convulsant treatment during cooling

All infants underwent single-channel aEEG using cross-brain C3-C4 electrode placement monitoring (CFM Olympic monitor, Natus, Seattle, USA) from before cooling, throughout the whole cooling treatment until post-rewarming. aEEG applies an algorithm to the raw EEG signal of time compression, high and low pass filtering and amplitude weighting onto a semi-logarithmic voltage

scale[16]. Sub-dermal needle electrodes (27G, Natus Medical Inc. Seattle, USA) were used for all infants without adverse effects including those who were cooled with a CoolCap covering the head. Clinically apparent seizures were treated, as well as prolonged (≥3min), single or repetitive electrical seizures or status epilepticus; seizure activity lasting ≥30min seen on the aEEG **and confirmed running the trace as single-channel EEG**. **aEEG** monitoring was used both to diagnose seizures and guide anticonvulsant treatment. A standard escalating AED protocol was used from 2006-2013: phenobarbitone bolus (20mg/kg)x1 or x2→ phenytoin (20mg/kg)x1→ clonazepam (100 microgram/kg)x1 or occasionally midazolam infusion at 0.1-0.4mg/kg/h[17]→ lidocaine infusion (loading dose of 2mg/kg, followed by continuous infusion at a rate of 6-2mg/kg/h)[18]. In one case, levitracetam and carbamazepine were used, and in another three cases, rectal paraldehyde (0.4ml/kg in dilution) successfully controlled refractory seizures. The **aEEG traces and single-channel EEG (when seizures were suspected), were** reviewed in real time for clinical decision-making as well as post-discharge for further analysis.

The standard rewarming rate **from 33.5-36.5°C** was 0.4°C/h. When aEEG **and** EEG confirmed seizure activity occurred during rewarming (n=14), rewarming was stopped and core temperature reduced by 1°C while AEDs were administered to control seizures followed by rewarming at a slower rate of 0.1-0.2°C/h. Five infants, who needed more than two AEDs to control seizures during cooling, were empirically rewarmed slowly. This clinical practice was developed in our newborn pig asphyxia model of post-hypoxic seizures [19], described in a clinical practice paper[20] and included in our local cooling guidelines.

After discharge from hospital, *"Children who presented with two or more unprovoked seizures at least 24 hours apart, or had a formal epilepsy syndrome diagnosis were classified as having epilepsy."*

Brain magnetic resonance imaging (MRI)

Infants were routinely scheduled for a brain MRI scan **between** 7 to 10 days. T1-, T2- and diffusion-weighted images were reviewed by a perinatal neurologist, our MR specialist (FC), who was only aware of the main diagnosis and age at scan. The scans were scored by summing up the severity grading developed by Rutherford et al[21] for basal ganglia and thalami (BGT, score 0-3), white matter (WM, score 0-3), cortex (CX, score 0-3), and posterior limb of internal capsule (PLIC, score 0-2) where 0 is no injury **and 3 is maximum injury**; giving a total maximum lesion injury score of 11. This is different from **how** the scoring was used in the nested MRI study from the TOBY cooling trial where cortical injury score was not included and BGT, WM and PLIC scores were not summated[21].

Clinical follow-up and data collection

Surviving children attended routine follow-up appointments at 6 weeks, 6, 12 and 24 months or more frequently as required. All children were scheduled for an assessment using the Bayley Scales of Infants and Toddler Development, 3rd edition (Bayley-III) (**mean** for normal =100, 1 standard deviation=15) at 18 months by a senior neurodevelopmental physiotherapist unaware of clinical details. Bayley-III motor composite and the average of cognitive and language composite (CLC) scores <85 were one of the criteria used to identify those children with adverse outcome. We have previously presented Bayley-III **cut-off** scores <85 to be comparable with Bayley-II scores of <70 **in identifying severe delay**[22]. Bayley-II <70 was the cut-off used for an adverse outcome in previously published cooling trials[22]. A formal diagnosis **of cerebral palsy(CP)** was made at 2 years. **Type of CP was classified according to the Surveillance of Cerebral Palsy in Europe** and graded using the Gross Motor Function Classification Scale (GMFCS): levels 3-5 were considered severe, grades 1-2 mild and a score of 0 was given to infants without CP. GMFCS grades 3-5 were used as an adverse outcome for the binary analysis, as in the earlier trials of TH.

Information about any post-neonatal seizures, EEG findings and medications was gathered from available local and regional outpatient appointments, relevant discharge notes from inpatient stays or the accident and emergency departments for children in this region since birth. In the earlier neonatal literature, receiving regular AEDs at the age of 2 years was used as a proxy definition of epilepsy[3; 4; 6]. We present this information in addition to defining epilepsy according **to ILAE**[8]. Information on seizures occurring after 2 years of age was collected for the 103 children born before 2012 (now aged up to 8 years).

When a binary outcome was used in the analysis, composite adverse outcome was death or disability defined as having at least one of: Bayley-III scores <85 for the average CLC or motor composite, severe CP (GMF**CS** 3-5), severe visual deficits or severe bilateral hearing loss. Epilepsy was not used as a marker of severe outcome for this study. Children who did not have Bayley assessments (n=13) were given an estimated binary outcome from the median value for the 134 survivors. On reviewing the neonatal data of these 13 children, this outcome was concordant with a low severity score at birth and needing no or only 1 AED in the neonatal period.

Statistical analysis

Non-parametric data were presented as median (interquartile range, IQR) as mostly non-normal distributions were identified in our data. Two groups comparison was undertaken using “N-1” chi square test since sample sizes were small or moderate.

Results

Betw**een 2006 -2013, 165** infants received TH; however, **on review of entry criteria** 14(8%) were found to have a normal aEEG background pattern (Continuous Normal Voltage;CNV) without seizure activity prior to cooling hence failing "entry criteria C". None seized, all had normal outcomes and they were not included in the main analyses. Their clinical, cooling and aEEG data are reported separately in tables 1 and 2 together with the 151 correctly recruited infants. Seventeen infants died (11%)(**13** before rewarming) of whom **16 seized**. At 2 years, 33 survivors(22%) had adverse developmental outcomes, hence death and disability combined was 33%. Outcome varied with the aEEG severity classification before 6h; 6% **had poor outcome** in the CNV+seizure group **and** 87% in the Flat Trace group (**table 2)**.

Epilepsy at 2 years and at 4-8 years

Of 134 survivors, only **8(6%)** presented with seizure activity between neonatal discharge and 2 years, fulfilling the ILA**E** epilepsy definition but only three infants (2%) were on AEDs (table 3). Of the 103 infants who reached 4-8 years, **6 more** fulfilled the ILAE epilepsy definition and four more, (7% of survivors) were now on AEDs. Five of the 14 with **epilepsy** had another diagnosis in addition to evidence of perinatal asphyxia **(table 3),** two with large intra- or extracerebral bleeds, two with suspected metabolic/genetic disorders and one a chromosome 15 (15q11.2) deletion. In summary, only 2% of the children were on regular AEDs at two years increasing to 7% at 4-8 years. A total of 14 children were diagnosed with epilepsy (**ILAE**) in early school age.

Early childhood seizures, other neurodevelopmental outcomes and MRI findings

Cerebral palsy

Twenty-two (15%) of 134 survivors were diagnosed with CP by 2 years, nine of whom had childhood seizures. Most children had mild forms of CP, being able to walk[23]. Children with CP were more likely to present with signs of seizures (9/14; 64%) than those who did not (13/120; 11%) ($p<0.001$). Details of the individual children (n=14) with post-neonatal seizures, their use of AEDs, CP grade, summated MRI scores and aEEG severity are presented in table 3.

Neonatal seizures and use of AEDs

Figure 1 outlines when infants seized from birth to discharge; 31 never had any documented seizures, 35 only seized pre-cooling, 32 only during cooling and 40 infants seized both before and during cooling. **The** majority, 113/151 (75%) of the infants had at least one seizure documented on **aEEG** and **single-channel** EEG before, during or after cooling. Seventy-five(50%) had single-channel

EEG seizures before cooling started. After day 5, all infants but 1 had stopped AEDs with no more seizures until discharge home or to local hospital at a median age of 9 days.

aEEG **and EEG** confirmed seizures in the neonatal period were treated according to protocol. Infants who received 0 or 1 AEDs had similar and good outcomes (table 3). The more AEDs needed neonatally, the worse the outcome. The number of AEDs was used as a proxy marker for predicting the rate of epilepsy and poor outcome (table 4). The number and duration of seizure episodes recorded **were** less predictive than the number of AEDs used for these outcomes.

MRI injury severity

Table 4 shows the summated MRI scores ranging from 0-11 and the regional distribution of MRI brain injury in the 14 patients presenting with epilepsy. MRI scans were abnormal in 12/14 infants. Patients 1-8 all had a BGT predominant pattern of injury. Those needing AEDs had more severe MRI scores than those not needing AEDs, median (IQR) 9(4-11) vs. 2(0-7) P<0.001. **The 8 infants who were diagnosed with epilepsy before the age of 2, had significantly worse median(IQR) MRI scores 9(8-11) than those who were diagnosed during childhood 2(0-4).**

Cognitive outcomes

Table 4 shows the individual Bayley-III scores and the **GMFCS** grades for the 14 children with epilepsy. Nine had CP, five with GMFCS grade 5. Four of these 5 children were on AEDs. Their Bayley-III CLC-scores were <55 (<-3SD). The 5th child had hypsarrythmia at 3 months but was not on medication by 3 years. These five children all had high MRI scores (8-11) and maximum BGT injury.

Of the remaining 9 children, five had Bayley-III CLC scores <80 and four had composite scores in the normal range (85-118). It was unexpected that the CLC in two children were as high as 77 and 72 when their MRI scores were 7 and 9 respectively.

Two infants had low MRI scores and unexpectedly poor outcomes. Child 11 developed mild 4-limb **dyskinetic** CP, a poor motor score of 76, CLC score of 74 **as well as** severe hearing loss. This child had one seizure during cooling and one after rewarming, treated with phenobarbitone. Pre-cooling aEEG pattern was burst suppression and it took 48 hours before the **background pattern** normalised, a poor prognostic factor[24] in addition to hypoglycaemia at birth of 1.3 mmol/L. Child 12 had a CLC of 78 and motor score of 61 but not CP. He had one episode of EEG confirmed clinical seizures during cooling, which responded quickly to phenobarbitone. His glucose at birth was low at 2.5mmol/L. **Pre-cooling** aEEG was burst suppression which did not recover to normal voltage for 72 hours. His **trachea-**

oesophageal fistula was operated on during cooling. A genetic syndrome is still being sought. **He presented with seizures** at 4 years and is **now seizure-free on** carbamazepine.

In summary, 10/14(71%) children with an epilepsy diagnosis up to the age of 8 years had adverse outcomes **in other domains in** comparison to 23/120(19%) of surviving infants without seizures.

Discussion

We have followed a 7-year clinical cohort of infants for at least 2 years and most for longer. All were treated with TH after moderate or severe perinatal asphyxia. Hypothermia is neuroprotective in particular for injury of hypoxic-ischemic origin if started within six hours of birth. Diagnoses other than HIE usually come to light after TH has started, and a clinical cohort will always include some infants with other **diagnoses** as presented here. Many centres do not use aEEG as a selection criterion for cooling and in a recent paper with only post-hoc aEEG analysis, 30% of cooled infants had a normal aEEG [25]. This means that many cooled cohorts are milder than those presented in the randomised trials and comparing outcomes between cohorts without severity classification of aEEG will be misleading. Using aEEG severity at entry is the best variable for classifying HIE severity when cooling starts[24; 25]. In an ideal world, all HIE infants should have multi-lead continuous EEG monitoring, but this is not feasible for most centres. In 2014 Glass et al[26] reported a US 3-centre retrospective study where infants had undergone continuous video-EEG (aEEG) starting at a median age of 9.5h(IQR 6.3-14.5h), i.e. after cooling started so these data cannot be compared with respect to seizures before cooling. They report a normal background EEG in 34 infants, excessive discontinuity in 20 and severe abnormality in 30 infants; 49% of their infants had clinical seizures before monitoring started. We cannot compare our background aEEG pattern with this study as our infants were monitored much earlier and those with a normal aEEG pattern before 6 hours(8%) were not included in the main analysis whereas Glass et al included all patients as they do not use EEG as a recruitment criterion. However, they report that 48% had seizures during or after cooling, very similar to us with 49% (table 3). Additionally, their cohort is of similar severity to ours.
Historically, and also used in the large cooling trials[3-5] neonatologists have used a pragmatic definition of epilepsy – the regular use of AEDs at the age of 2 years as well as at 5-7 years follow-up [4]. **We now** present results **adding** the newer ILAE epilepsy definition[8] which doubles the number of cooled children having a diagnosis of epilepsy. In our cohort only 2% of children were on AEDs at 2 years but this had increased to 7% by 4-8 years. A low seizure rate at 2 years amongst cooled infants was also found in Glass et al´s study[26]. In the major trials the number of infants on AEDs at 18 months in the cooled group was 15% (CoolCap), 17% (NICHD) and 10% (TOBY) and the corresponding values in the standard treatment groups were 16%, 22% and 14%. In contrast to our

data, school-age follow-up in the NICHD trial[7] found that only 40% of the cooled and 50% of the non-cooled children who had been on regular AEDs at two years continued to be so at the age of 6-7 years. It is unknown whether different post-discharge AED prescribing practices in the US and Europe after neonatal seizures **has affected these numbers**. The clinical severity of HIE assessed by entry criteria A in our cohort, was milder with regard to Apgar scores compared to the first cooling trials[3-5]. In addition, when comparing the aEEG voltage pattern at entry (criterion C), the proportion with a severe pattern was 60% in TOBY, 40% in CoolCap and 34% in our cohort indicating we have a milder cohort (Table 5, appendix). **However, as aEEG was a new tool to the 60 NICU's recruiting for the named trials, ideally** their aEEG severity scoring should be validated. At that time, many aEEGs were paper traces running at 6cm/h without concurrent raw EEG traces unlike today's digital aEEGs. Therefore, **re-analysis of the entry aEEG in the CoolCap and TOBY trials has been difficult. Clinical seizures only will underestimate the seizure burden, a seizure study[27] in term infants recorded simultaneous 16 lead EEG and video for 72h and only 9% of electrographic seizures were accompanied by documented clinical manifestations. Despite our milder cohort, the** incidence of neonatal seizures (clinical and aEEG) was comparable to CoolCap and TOBY. Overall, 83% of infants had seizures in the CoolCap trial (recruiting 1999-2002), 77% in the TOBY trial (recruiting 2002-2006) and 77% in this study recruiting 2006-2013 **(Table 5).**

The duration of continuous recording is long in our cohort (≥78h) and in particular we start **aEEG** early from around 1h hence we detect seizures **early. It is, however, interesting that** 43% of infants with neonatal seizures before 6h in our study **did** not have any further seizures **which carried the same good prognosis as the group who never had any seizures at all.**

After having investigated the safety of the 3-day CoolCap protocol before the trial commenced[10] , we developed a local cooling treatment protocol where all at-risk infants are passively cooled while being investigated before deciding to start active cooling. Passive cooling was started at a median **age of 0.75 hours**. We found better motor, but not cognitive outcome in infants **who** starting cooling early (<3 hours) as compared to between 3-6 hours[28]. No child was ever hyperthermic (>38°C before cooling (Table 1)). Our ventilator strategy is to give as little oxygen as possible to keep normal saturations, keep pCO_2 values in the high normocapneic range[29] avoiding hypotension[29]. We use aEEG monitoring to recruit to TH, to classify infants into severity groups and to direct seizure care; we give AEDs for both clinical and electrographic seizures with a low treatment threshold. We suggest that be able to apply and read an aEEG at a basic level to assess background voltage and aEEG patterns with **single-channel** EEG for seizure occurrence should be a necessary skill in neonatology. We can only speculate that the combined brain oriented clinical management and early cooling have reduced the rates of post-neonatal epilepsy. On the other hand, our cohort is

somewhat milder than the large trials, and severe brain injury is likely less. Experimentally, Alistair Gunn claims that the frequency and amplitude characteristic of seizures while cold is not harmful *per se*[30]. In a Dutch pre-cooling study, a lower incidence of childhood epilepsy was seen in children whose electrographic and clinical seizures were treated compared to only treating clinical seizures[31]. However, our study cannot provide direct evidence that treating electrographic seizures benefits infants long-term; neither do we know whether only treating acutely and not giving maintenance AEDs after discharge is beneficial.

Most AEDs are metabolised in the liver and drug metabolism is generally reduced at low core temperature[32]. There has been no change in AED regimes since TH was introduced in our cooling centre except for reduced use of paraldehyde, which is no longer available and reduced dosages of lidocaine[33]. It is reasonable to suggest that prolonged higher AED plasma levels are present in cooled[34] compared to normothermic infants although we do not know of the clinical impact.

Neonatal brain MRI has proven useful in defining injury severity in HIE and understanding causation of neonatal seizures[35; 36]. Children diagnosed with epilepsy in our study had a high MRI lesion load consistent with Martinez-Biarge *et al*'s finding that "30-40% of non-cooled children with epilepsy had basal ganglia and thalamic lesions"[37]. Children who develop CP after perinatal asphyxia are at high risk of developing epilepsy[38]. Studies investigating epilepsy in children with CP have reported that 35-43% developed seizures in the post-neonatal period[39-42], very similar to our cohort where 9 of 23 infants with CP(39%) developed seizures. However, variations in seizure definition and severity of HIE make direct comparisons between studies a challenge.

Ten of the 14 children with epilepsy had a Bayley-III CLC score <85.This is consistent with a Swedish study showing that epilepsy is associated with decreased cognitive function in children with CP[39].

From our study of infants cooled for neonatal encephalopathy, we conclude that we have a very low incidence of childhood epilepsy at 2 years but this rate increases as the children reached 4-8 years. Five infants had other factors that came to light during or after cooling therapy including a defined chromosomal abnormality and two suspected genetic or metabolic conditions. The encephalopathy seen may not be only due to hypoxic-ischemic causes. Long-term follow-up is needed to determine final epilepsy rates and collaborative studies on optimal treatment regimens as the children mature.

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Disclosures

There are conflicts of interest to disclose. We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Table 1 Demographic and cooling related information on all 165 infants

Values are median (IQR), unless indicated.	n=151 correctly recruited to TH - abnormal aEEG background voltage prior to cooling	n=14 incorrectly recruited to TH– normal aEEG background voltage prior to cooling.
Outborn %	47	57
Gestational age in weeks	40.3 (38.3-40.7)	40.15 (39.3-40.8)
Male %	62	57
Birth weight (centile)	25 (9-75)	63 (31-75)
Apgar score at 10 minutes	6 (4-8)	7 (6-8)
Worst pH within the 1st hour after birth	6.94 (6.82-7.07)	6.94 (6.90-6.99)
Worst base deficit within the 1st hour after birth	15.5 (12-20.5)	14.8 (12.5-16.1)
Number of infants with seizures before cooling (%)	84 (56)	0 (0)
Passive cooling commenced (age in hours)	0.75 (0.16-3.43)	0.94 (0.14-3.23)
Active cooling commenced (age in hours)	3.78 (2.02-5.83)	5.41 (3.77-6.07)
Age when the target temperature was achieved (hours)	4.75 (2.87-7.23)	5.22 (4.95-6)
Highest temperature (°C) before active cooling commenced	34.6 (33.0-36.1)°C	35.4 (34.1-36.0)°C
Deaths number (%)	17 (11%)	0 (0%)

aEEG: amplitude-integrated EEG TH: therapeutic hypothermia

Table 2 shows the aEEG data, in the second column, classified using the voltage method from a **single-channel** aEEG[13] and in the third column classified from the aEEG background pattern[24] with increasing aEEG severity before active cooling started in all 165 infants. The number of patients with an adverse outcome (binary) for each background pattern category is shown in the fourth column. The fifth column lists how many infants had any type of post neonatal seizures in each aEEG pattern category.

An adverse outcome is defined as death or disability by 18-24 months of age (see methods). A good outcome is **defined as having no adverse outcomes.**

aEEG pattern (criterion C) prior to active cooling in 165 infants	Voltage classification pattern Number (percentage) n=165 (100%)		aEEG background pattern classification Number (percentage) n=165 (100%)		Number of children with an adverse developmental outcome in each pattern group	Number of children with childhood seizures in each aEEG pattern group
	Normal voltage	14 (8%)	Continuous Normal Voltage (CNV) only*	14 (8%)	0(0 %)	0(0 %)
	Moderately abnormal voltage	129(78 %)	CNV With electrographic Seizures	18 (12 %)	1(6 %)	0 (0 %)
			Discontinuous Normal Voltage	38 (23 %)	8(21 %)	1 (3 %)
			Burst Supression	73 (44 %)	24(33 %)	9 (12 %)
	Severely abnormal voltage	22(14 %)	Low Voltage	7 (4 %)	4(57 %)	1(14 %)
			Flat Trace	15 (9 %)	13(87 %)	3 (20 %)

*When the aEEG was reassessed off line, 14 infants (8% of the cohort) had a normal aEEG background pattern (CNV) without seizures and did not fulfill the third criterion for therapeutic hypothermia treatment. *These 14 are excluded in some of the **later** analysis.

Table 3: A summary of the neurology information in the 14 children who were diagnosed with epilepsy according to the definition by the International League against Epilepsy (ILAE) at 2 years or by 4-8 years

Patient number	MRI score (0-11)	Affected brain regions MRI	GMFCS score (0-5)	Severe hearing loss	ILAE definition epilepsy age ≤2 years (on set age)	On AEDs at 2 years	ILAE definition epilepsy after 2 years	On AEDs at 4-8 years	Bayley-III composite cognitive-language /motor scores at 18-24 months	Number of AED Used	*On set aEEG pattern prior to cooling	TTNT aEEG pattern	Other known diagnosis
1	11	BGT, PLIC, WM and Cortex	5	yes	1 month	✓			<55/<46	3	FT	>84h	major intracranial hemorrhage
2	11	BGT, PLIC, WM and Cortex	5	no	1 month	✓			<55/<46	5	FT	>84h	
3	10	BGT, PLIC, WM and Cortex	5	no	6 months	✓			<55/<46	4	LV	>84h	
4	9	BGT, PLIC, WM and Cortex	2	no	1 year?			✓	72/79	4	BS	82h	cooled at 12h delayed decision
5	9	BGT, WM, PLIC and Cortex	5	no	4 months#				<55/<46	5	BS	169h	
6	8	BGT, WM, PLIC and Cortex	5	no			2.5	✓	<55/<46	2	BS	21h	
7	7	BGT, PLIC, WM and Cortex	0	yes			5.5		77/76	1	FT	>84h	metabolic^

8	4	WM, BGT, PLIC	0	no			7	✓	Global delay, not tested	0	BS	8h	chromosomal^^
9	4	Cortex, WM	0	yes			6.5		93/97	2	BS	36h	
10	3	Cortex, WM	1	no	6 months				94/82	5	BS	>84h	major intracranial hemorrhage
11	2	WM	1	yes			4.5		74/76	1	BS	48h	
12	2	WM	0	no			4	✓	78/61	1	BS	>84h	chromosomal¤
13	0	**none**	1	No	<1 year				91/82	1	BS	15h	
14	0	**none**	0	No	1 year				118/112	1	DNV	0h	

*Time to recovery of the aEEG trace to a normal (for being cooled) pattern. TTNT >48h predicts poor outcome [24]

AEDs: Anti-Epileptic Drugs, MRI: Magnetic Resonance Imaging

hypsarrythmia, ^metabolic disease under investigation, ^^ chromosome deletions (15q11.2), ¤ chromosomal condition under investigation

Table 4: The number of anti-epileptic drugs (AEDs) used in the neonatal period in relation to neuro-developmental outcome at 18 months in 151 infants undergoing therapeutic hypothermia.

Number of AEDs used until neonatal discharge	Total number of infants in the group	Number with a good outcome	Number of survivors with adverse outcome	Number died	Total % with adverse outcome*	Escalating drug protocol for treating seizures (clinical with or without aEEG/EEG confirmation) in cooled infants
0	38	31	6	1	7/38 18%	none
1	60	46	8	6	14/60 23%	20/kg phenobarbitone (1 or 2 doses)
2	20	11	5	4	9/20 45%	+ 20mg/kg phenytoin (1 dose)
3	19	9	6	4	10/19 53%	+ 100 mg/kg clonazepam (1/2 doses)
4	12	4	6	2	8/12 67%	+ continuous lidocaine or midazolam infusion or both
5	2	0	2	0	2/2 100%	+ paraldehyde 3mg/kg up to 6 hourly
Total number of infants	151	101	33	17	50/151 33%	

*An adverse outcome was defined as death or disability; disability as defined as having a Bayley-III scores <85 for at least one of the average of Cognitive and Language composite or Motor Composite and/or cerebral palsy with a Gross Motor Function Score of 3-5 or severe visual deficits or bilateral hearing loss requiring hearing aids

Table 5: aEEG background voltage before cooling in two multicenter clinical trials and this study (Appendix)

Studies	CoolCap	TOBY	This study
Normal aEEG background (voltage criteria)[13]	4%	0%	8%
Normal aEEG background with EEG seizures	6%	0%	12%
Moderately abnormal aEEG background	54%	40%	66%
Severely abnormal aEEG background	36%	60%	14%
Seizures prior to decision to give therapeutic hypothermia at 6 hours	59%*	56%$	54%$

*Pre-randomisation **aEEG and single-channel EEG** with seizures $Clinical and/or aEEG documented seizures